

The Effect of Substitution at C-2 of D-Glucose 6-Phosphate on the Rate of Dehydrogenation by Glucose 6-Phosphate Dehydrogenase (from Yeast and from Rat Liver)

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1. The deoxyfluoro-D-glucopyranose 6-phosphates are substrates for both yeast and rat liver glucose 6-phosphate dehydrogenase. 2. The V_{max} values (relative to D-glucose 6-phosphate) were determined for a series of D-glucose 6-phosphate derivatives substituted at C-2. The V_{max} values decreased with increasing electronegativity of the C-2 substituent. This is consistent with a mechanism involving hydride-ion transfer. 3. 2-Deoxy-D-arabino-hexose 6-phosphate (2-deoxy-D-glucose 6-phosphate) showed substrate inhibition with the yeast enzyme but not with the rat liver enzyme. 4. 2-Amino-2-deoxy-D-glucose 6-phosphate (D-glucosamine 6-phosphate) was a substrate for the yeast enzyme but a competitive inhibitor for the rat liver enzyme. 5. Lineweaver–Burk plots for the D-glucose 6-phosphate derivatives with yeast glucose 6-phosphate dehydrogenase were biphasic.

Although the inductive effect of substituents on the rate of dehydrogenation by horse liver alcohol dehydrogenase has been studied (Blomquist, 1966; Tsai, 1968), the effect on the rate of dehydrogenation by glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate–NADP⁺ oxidoreductase, EC 1.1.1.49) has not been examined, because suitable derivatives have not previously been available. The enzymic synthesis of the deoxyfluoro-D-glucopyranose 6-phosphates and 2-chloro-2-deoxy-D-glucose 6-phosphate (Bessell & Thomas, 1973) has now provided suitable derivatives. The dehydrogenation occurs at the anomeric carbon atom (C-1) of glucose 6-phosphate, and hence the inductive effect on the dehydrogenation reaction of a substituent on the vicinal carbon atom can be studied by using C-2-substituted D-glucose 6-phosphates. The series of compounds D-glucose 6-phosphate, D-glucosamine 6-phosphate, 2-deoxy-D-arabino-hexose 6-phosphate, 2-chloro-2-deoxy-D-glucose 6-phosphate and 2-deoxy-2-fluoro-D-glucose 6-phosphate have been studied as substrates for both yeast and rat liver glucose 6-phosphate dehydrogenase.

Experimental

Glucose 6-phosphate dehydrogenase (EC 1.1.1.49; type 1 purity from yeast; 350 units/mg), ATP and NADP⁺ were purchased from Boehringer Corp. (London) Ltd., London W.5, U.K. Hexokinase (EC 2.7.1.1; C-301 from yeast; 200–300 units/mg), D-glucosamine 6-phosphate and 2-deoxy-D-arabino-hexose 6-phosphate were obtained from Sigma (Lon-

don) Chemical Co., London S.W.6, U.K. Deoxyhalogeno-D-glucose 6-phosphates were prepared from the corresponding deoxyhalogeno-D-glucose and ATP by using yeast hexokinase (Bessell & Thomas, 1973).

Preparation of glucose 6-phosphate dehydrogenase from rat liver

Rat liver glucose 6-phosphate dehydrogenase was purified by an extension of the method of Dickens & Glock (1951). Male Wistar rats (approx. 200g) were starved for 24 h and fed on a synthetic fat-free diet for 3 days. The rats were then killed by neck dislocation and their livers removed and cooled in ice. Stage 1. The livers (30 g) were homogenized with sufficient cold KCl (1.2%, w/v) to give a 20% (w/v) suspension. The resulting suspension was centrifuged at 100000g for 60 min and the supernatant retained. Stage 2. The supernatant from Stage 1 was adjusted to pH 4.5 by the slow addition of 0.5 M-acetic acid. The precipitate which formed was removed by centrifuging at 3000g for 30 min. The resulting supernatant was immediately adjusted to pH 7.5 by the addition of 0.1 M-NaOH. The precipitate which formed was again removed by centrifugation. Stage 3. (NH₄)₂SO₄ was added with mechanical stirring to the Stage 2 supernatant to give a 40%-saturated solution. After standing for 1 h the precipitate was removed by centrifuging at 3000g for 30 min. Additional (NH₄)₂SO₄ was added to the supernatant as before to give a 60%-saturated solution. The solution was left for 2 h and the precipitate was then separated by centrifugation as

described above. Stage 4. The fraction precipitating between 40 and 60% saturation from Stage 3 was dissolved in 0.01 M-Tris-HCl buffer, pH 7.5 (approx. 15 ml), and dialysed twice for 24 h with the same buffer (total volume 5 litres). The dialysis residue (approx. 20 ml) was applied to a column (20 cm × 2 cm) of DEAE-cellulose (Whatman DE-52) previously equilibrated with the same buffer and containing 0.1 mM-NADP⁺. Elution with a linear gradient (0.01 M-tris-HCl buffer, containing 0.1 mM-NADP⁺, pH 7.5, 200 ml; 0.01 M-Tris-HCl buffer containing 0.5 M-KCl and 0.1 mM-NADP⁺, pH 7.5, 200 ml) at a flow rate of 20 ml/h gave a purified preparation of glucose 6-phosphate dehydrogenase.

The enzyme solution was concentrated by vacuum dialysis by using a Sartorius membrane (Sartorius-membranfilter G.m.b.H., Göttingen, Germany).

This preparation was used in all subsequent experiments with the rat liver enzyme. Although not of a high specific activity (0.5 unit/mg) the preparation was free of glucose phosphate isomerase (EC 5.3.1.9) and phosphogluconate dehydrogenase (decarboxylating) (EC 1.1.1.44) activities. Crystalline glucose 6-phosphate dehydrogenase has been prepared from rat liver with a specific activity of 128 units/mg (Matsuda & Yugari, 1967).

Initial-rate measurements

Glucose 6-phosphate dehydrogenase activity was assayed in 0.1 M-Tris-HCl buffer, pH 7.5, in the absence of Mg²⁺ by using the standard procedure of observing the rate of increase of E_{340} . For the determination of the kinetic constants, given in Tables 1 and 2, the concentrations of the glucose 6-phosphate derivatives used were in the range from one-tenth to twice the K_m value. Higher concentrations could not be used because sufficient material was not available. The concentrations of NADP⁺ used were in the range from one to ten times the K_m value.

Rates for yeast glucose 6-phosphate dehydrogenase were measured by using a Pye Unicam SP.500 monochromator with a Gilford model 220 absorbance indicator, the output of which was connected to a Honeywell strip-chart recorder. The full-scale deflexion of the recorder was set to correspond to a ΔE_{340} of 0.1. Rates for rat liver glucose 6-phosphate dehydrogenase were measured by using a Cary model 16KC kinetic spectrophotometer, the output of which was connected to a Varian strip-chart recorder model G-2510. The full-scale deflexion of the recorder was set to correspond to a ΔE_{340} of 0.01 or 0.02. The cell compartment was thermostatically maintained at 30°C in both cases. Silica cells (1 cm light-path) were filled with all components of the reaction mixture except the enzyme, in a total volume of 2.9 ml. The reaction was initiated with enzyme (0.1 ml) suitably diluted with 0.1 M-Tris-HCl buffer, pH 7.5.

Data processing

Values of K_a , K_b , K_{ia} and V_{max} in eqn. (1) were obtained by using a computer program written by Cleland (1963). K_a , in this case, is the Michaelis constant for the glucose 6-phosphate derivative, K_b is the Michaelis constant for NADP⁺ and K_{ia} is the inhibition constant for the glucose 6-phosphate derivative. The program was slightly modified by Dr. L. I. Hart to run on the London University CDC 6600 computer.

$$v = \frac{V_{max} [A][B]}{K_{ia} K_b + [A]K_b + [B]K_a + [A][B]} \quad (1)$$

Values of K_i , K_m and V_{max} in eqn. (2) for linear competitive inhibition were obtained by using a computer program written by Cleland (1963):

$$v = \frac{V_{max} [S]}{K_m (1 + [I]/K_i) + [S]} \quad (2)$$

For substrate inhibition data were fitted to eqn. (3):

$$v = \frac{V_{max} [S]}{K_m + [S] + [S]^2/K_i} \quad (3)$$

Values of K_m , K_i and V_{max} were obtained from eqn. (3) by using a pH-function curve-fitting program written by Cleland (Cleland, 1967, eqn. 25). This program was modified by Dr. L. I. Hart to fit eqn. (3) by using v^4 weights, but being a non-iterative procedure the best final values may not have been obtained.

Results

Most of the D-glucose 6-phosphate derivatives used were substrates for yeast glucose 6-phosphate dehydrogenase. The kinetic constants were obtained by using eqn. (1) and are given in Table 1. A double-reciprocal plot for 2-deoxy-2-fluoro-D-glucose 6-phosphate is shown in Fig. 1. 2-Deoxy-2,2-difluoro-D-arabino-hexose 6-phosphate and 2-deoxy-2-fluoro-D-mannose 6-phosphate, prepared *in situ* from the corresponding D-hexose and ATP by using yeast hexokinase, were not substrates for yeast glucose 6-phosphate dehydrogenase. The kinetic constants for the D-glucose 6-phosphate derivatives, when rat liver glucose 6-phosphate dehydrogenase was used, were also obtained by using eqn. (1) and are given in Table 2. A double-reciprocal plot for 2-chloro-2-deoxy-D-glucose 6-phosphate is shown in Fig. 2. D-Glucosamine 6-phosphate was not a substrate but was a competitive inhibitor, and the K_i value, determined by using eqn. (2), is 0.75 ± 0.05 mM. The apparent K_m value for D-glucose 6-phosphate (NADP⁺ concentration 0.16 mM) was obtained from the competitive-inhibition results by using eqn. (2) and is 0.031 ± 0.002 mM. The competitive inhibition by D-glucosamine 6-phosphate is shown by a double-reciprocal plot in Fig. 3. Although inhibition at high

Table 1. Kinetic constants for yeast glucose 6-phosphate dehydrogenase

For experimental details see the text. The values are means \pm s.e.m.

Compound	K_a (mM)	K_{ia} (mM)	K_b (μ M) (NADP ⁺)	Relative V_{max} .
D-Glucose 6-phosphate	0.070 \pm 0.017	0.083 \pm 0.036	15 \pm 5	1
2-Deoxy-2-fluoro-D-glucose 6-phosphate	1.18 \pm 0.15	0.41 \pm 0.18	43 \pm 6	0.016
2-Chloro-2-deoxy-D-glucose 6-phosphate	0.80 \pm 0.13	2.10 \pm 0.63	22 \pm 4	0.33
2-Deoxy-D-arabino-hexose 6-phosphate	15 \pm 10	—	32 \pm 7	0.64
D-Glucosamine 6-phosphate	3.99 \pm 0.77	1.45 \pm 0.74	41 \pm 11	0.04
3-Deoxy-3-fluoro-D-glucose 6-phosphate	3.04 \pm 0.75	2.91 \pm 1.38	45 \pm 16	0.12
4-Deoxy-4-fluoro-D-glucose 6-phosphate	1.10 \pm 0.19	0.19 \pm 0.16	35 \pm 8	0.12

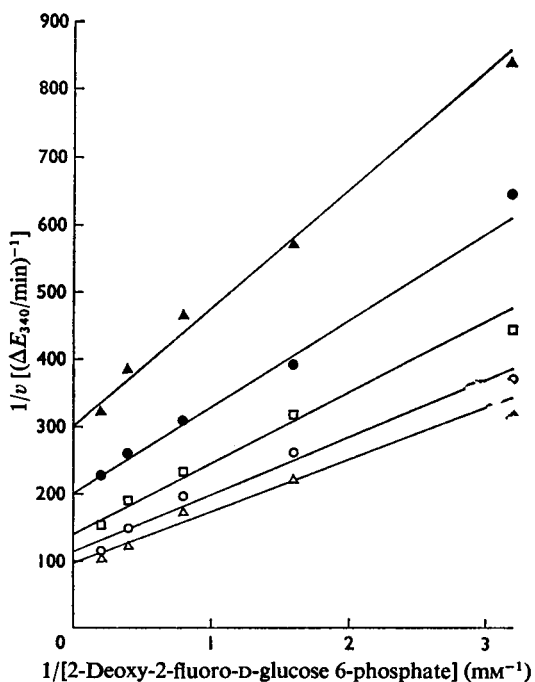


Fig. 1. Yeast glucose 6-phosphate dehydrogenase: variation of the reciprocal of the initial rate with the reciprocal of the 2-deoxy-2-fluoro-D-glucose 6-phosphate concentration for several constant NADP⁺ concentrations

For experimental details see the text. NADP⁺ concentrations used were: ▲, 12.5 μ M; ●, 25 μ M; □, 50 μ M; ○, 100 μ M; △, 200 μ M.

concentrations of glucose 6-phosphate was not observed with either yeast or rat liver glucose 6-phosphate dehydrogenase, inhibition was observed with high concentrations of 2-deoxy-D-arabino-hexose 6-phosphate with the yeast enzyme. This observation

has not been previously reported, although the K_m value for 2-deoxy-D-arabino-hexose 6-phosphate for yeast glucose 6-phosphate dehydrogenase has been determined (Greiling & Kisters, 1965). A value (4.5 mM) lower than the value of 15 \pm 10 mM reported in the present paper was given. The initial velocities obtained from various concentrations of 2-deoxy-D-arabino-hexose 6-phosphate at a constant concentration of NADP⁺ fitted eqn. (3). The values obtained for the apparent K_m and K_i at different NADP⁺ concentrations are given below the double-reciprocal plot in Fig. 4. The double-reciprocal plot at various NADP⁺ concentrations and constant 2-deoxy-D-arabino-hexose 6-phosphate concentrations is shown in Fig. 5. Straight-line plots could be obtained only at high and low concentrations of 2-deoxy-D-arabino-hexose 6-phosphate, i.e. when the initial velocities were low. At medium concentrations, when the initial velocities were maximal, deviation from linearity was observed. The initial velocities obtained therefore do not fit eqn. (4) for uncompetitive substrate inhibition (Cleland, 1970):

$$v = \frac{V_{max} [A][B]}{K_{ia} K_b + [A]K_b + [B]K_a + [A][B] + [A][B]^2/K_i} \quad (4)$$

The K_m value for 2-deoxy-D-arabino-hexose 6-phosphate, quoted in Table 1, was computed by using eqn. (1). The K_m value for NADP⁺ and the relative V_{max} were estimated graphically. Initial velocities obtained from concentrations of 2-deoxy-D-arabino-hexose 6-phosphate (2.5–10 mM) and NADP⁺ (0.025–0.20 mM) were used because straight lines could be obtained from double-reciprocal plots in this concentration range.

If the double-reciprocal plot for 2-deoxy-2-fluoro-D-glucose 6-phosphate with yeast glucose 6-phosphate dehydrogenase (see Fig. 1) is examined it shows that although one straight line can be drawn through the points a more exact fit would be obtained if two straight lines were drawn, one through the points at high 1/[S] values and one with a greater slope through the points at low 1/[S] values. These two straight lines

Table 2. Kinetic constants for rat liver glucose 6-phosphate dehydrogenase

For experimental details see the text. Values are means \pm S.E.M.

Compound	K_a (mM)	K_{ia} (mM)	K_b (μ M) (NADP ⁺)	Relative V_{max} .
D-Glucose 6-phosphate	0.010 \pm 0.003	0.026 \pm 0.018	9.3 \pm 2.4	1
2-Deoxy-2-fluoro-D-glucose 6-phosphate	1.94 \pm 0.44	1.72 \pm 1.69	8.6 \pm 5.3	0.027
2-Chloro-2-deoxy-D-glucose 6-phosphate	0.82 \pm 0.05	0.69 \pm 0.20	6.9 \pm 0.6	0.89
2-Deoxy-D-arabino-hexose 6-phosphate	1.39 \pm 0.24	5.19 \pm 3.66	3.8 \pm 1.6	0.055
3-Deoxy-3-fluoro-D-glucose 6-phosphate	3.93 \pm 0.83	—	8.4	0.25
4-Deoxy-4-fluoro-D-glucose 6-phosphate*	3.0	—	—	0.4

* The kinetic constants for this compound are only approximate values because insufficient amounts were available for an accurate determination.

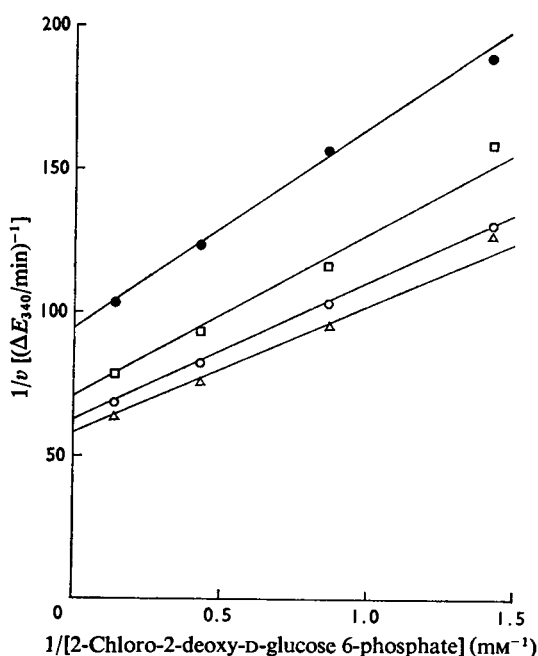


Fig. 2. Rat liver glucose 6-phosphate dehydrogenase: variation of the reciprocal of the initial rate with the reciprocal of the 2-chloro-2-deoxy-D-glucose 6-phosphate concentration for several constant NADP⁺ concentrations

For experimental details see the text. NADP⁺ concentrations used were: ●, 9.6 μ M; □, 19.2 μ M; ○, 48 μ M; △, 96 μ M.

would intersect and indicate an abrupt transition, although more experimental points would be needed to verify this. Transitions between two linear sections were observed in the double-reciprocal plots for all of the D-glucose 6-phosphate derivatives when the

reciprocal of the initial velocity was plotted against the reciprocal of the sugar phosphate concentration.

These transitions were found to be more marked with 2-deoxy-2-fluoro-D-glucose 6-phosphate (see Fig. 1) and D-glucosamine 6-phosphate, which had low relative V_{max} values, and also with 2-deoxy-D-arabino-hexose 6-phosphate (see Fig. 4). The plot against the reciprocal of the NADP⁺ concentration also showed this transition in a few cases.

This type of transition has been observed with yeast glucose 6-phosphate dehydrogenase when the reciprocal of the initial velocity was plotted against the reciprocal of the D-glucose 6-phosphate concentration (Rutter, 1957; Anderson *et al.*, 1968) and also with glucose 6-phosphate dehydrogenase from sweet potato (Muto & Uritani, 1970). This transition is thought to be due to negative co-operativity between the binding sites of subunits (Dalziel & Engel, 1968; Koshland & Neet, 1968). The change in slope results from the fact that 'sequential binding of ligand produces successively new sites of lower affinity but higher turnover number' (Conway & Koshland, 1968). The apoenzyme of yeast glucose 6-phosphate dehydrogenase exists as a dimer, but the NADP⁺-enzyme complex exists as a tetramer (Yue *et al.*, 1969). These transitions could be due to negative co-operativity between the four D-glucose 6-phosphate-binding sites as saturation of the enzyme by D-glucose 6-phosphate is approached, and the transition is more marked at low enzyme velocities.

Although these transitions were observed with yeast glucose 6-phosphate dehydrogenase they were not observed with the rat liver enzyme (see Fig. 2).

2-Deoxy-2-fluoro-D-glucose, 2-deoxy-2-fluoro-D-mannose and 2-deoxy-2,2-difluoro-D-arabino-hexose are all good substrates for yeast hexokinase (Bessell *et al.*, 1972). Since the products are either not substrates (2-deoxy-2-fluoro-D-mannose 6-phosphate, 2-deoxy-2,2-difluoro-D-arabino-hexose 6-phosphate) or a poor substrate (2-deoxy-2-fluoro-D-glucose 6-phosphate) for yeast glucose 6-phosphate dehydro-

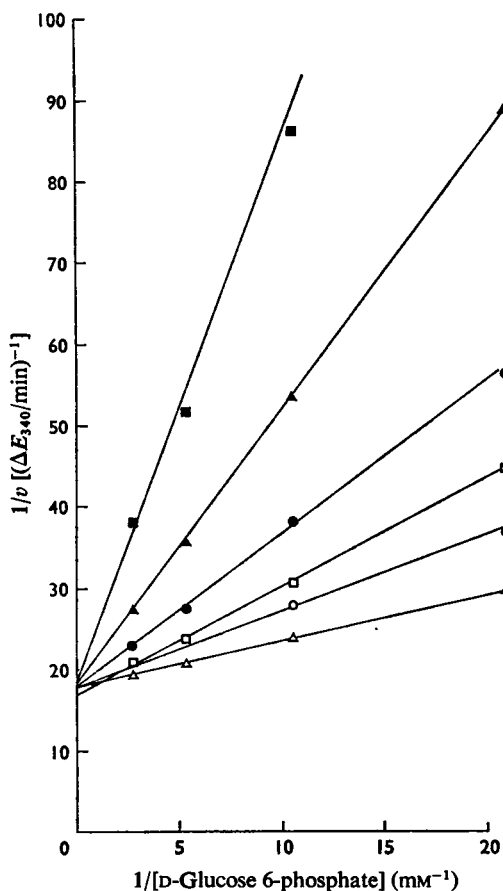


Fig. 3. Competitive inhibition of rat liver glucose 6-phosphate dehydrogenase by D-glucosamine 6-phosphate

The variation of the reciprocal of the initial rate is plotted against the reciprocal of the D-glucose 6-phosphate concentration for several constant concentrations of D-glucosamine 6-phosphate: ■, 8 mM; ▲, 4 mM; ●, 2 mM; □, 1 mM; ○, 0.5 mM; △, none. For experimental details see the text.

genase the effect of the fluorinated D-hexoses on the coupled enzyme system (hexokinase-glucose 6-phosphate dehydrogenase) was determined by measuring the competitive inhibition of D-glucose phosphorylation. The K_i values obtained by using eqn. (2) are for 2-deoxy-2-fluoro-D-glucose, 0.22 ± 0.03 mM, for 2-deoxy-2-fluoro-D-mannose, 0.20 ± 0.02 mM and for 2-deoxy-2,2-difluoro-D-arabino-hexose, 0.34 ± 0.06 mM. These values agree reasonably well with the K_m values obtained with yeast hexokinase (Bessell *et al.*, 1972) and show that these compounds are good

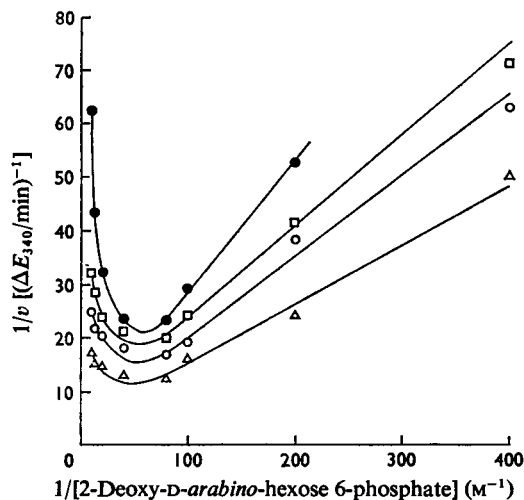


Fig. 4. Inhibition of yeast glucose 6-phosphate dehydrogenase at high 2-deoxy-D-arabino-hexose 6-phosphate concentrations

The variation of the reciprocal of the initial rate is plotted against the reciprocal of the 2-deoxy-D-arabino-hexose 6-phosphate concentration for several constant concentrations of NADP^+ ; ●, 25 μM ; □, 50 μM ; ○, 100 μM ; △, 200 μM . For $[\text{NADP}^+] 200 \mu\text{M}$, $K_m = 11.5 \pm 0.6$ mM, $K_i = 61.6 \pm 0.6$ mM; for $[\text{NADP}^+] 100 \mu\text{M}$, $K_m = 12.1 \pm 0.7$ mM, $K_i = 48.2 \pm 0.6$ mM; for $[\text{NADP}^+] 50 \mu\text{M}$, $K_m = 17.6 \pm 0.6$ mM, $K_i = 30.8 \pm 0.5$ mM; for $[\text{NADP}^+] 25 \mu\text{M}$, the data did not fit eqn. (3) sufficiently well to give values of K_m and K_i . For experimental details see the text.

inhibitors of glucose phosphorylation *in vitro*. 6-Deoxy-6-fluoro-D-glucose (Bessell *et al.*, 1971) is a competitive inhibitor of yeast hexokinase and the K_i value, determined by using eqn. (2), was 12.7 ± 1.5 mM. 6-Deoxy-6-fluoro-D-glucose is not an inhibitor of yeast glucose 6-phosphate dehydrogenase.

Discussion

Yeast glucose 6-phosphate dehydrogenase is fairly specific as far as substrate configuration is concerned. Only the β -anomer of D-glucopyranose 6-phosphate is a substrate for the enzyme (Salas *et al.*, 1965) and neither the C-2 epimer, D-mannose 6-phosphate, nor the C-3 epimer, D-allose 6-phosphate, are substrates or inhibitors (Együd & Whelan, 1963). The C-4 epimer, D-galactose 6-phosphate, however, is a substrate (Együd & Whelan, 1963), and shows that the stereochemistry at C-4 is not so important. Inversion of both C-2 and C-3 of the D-glucose configuration gives the D-altro configuration,

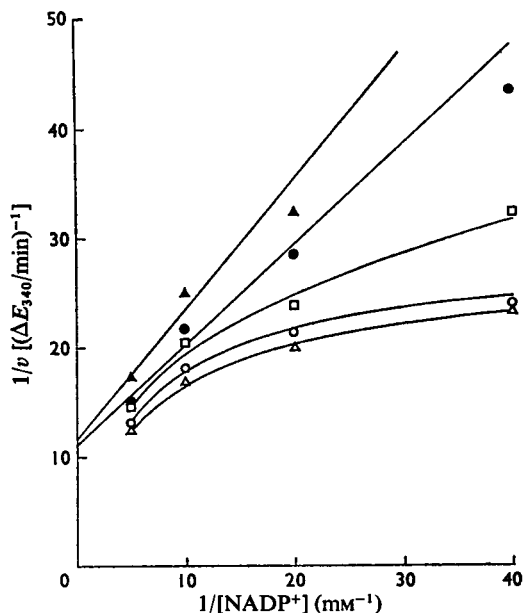


Fig. 5. Yeast glucose 6-phosphate dehydrogenase: variation of the reciprocal of the initial rate with the reciprocal of the $NADP^+$ concentration for several concentrations of 2-deoxy-D-arabino-hexose 6-phosphate

For experimental details see the text. 2-Deoxy-D-arabino-hexose 6-phosphate concentrations used were: ▲, 100mM; ●, 75mM; □, 50mM; ○, 25mM; Δ, 12.5mM.

and it is thus surprising that D-altrose 6-phosphate is a substrate (Együd & Whelan, 1963). This might be explained if the conformation of D-glucose 6-phosphate in solution is considered. D-Glucose 6-phosphate should exist in aqueous solution to a very large extent in the pyranose form with each anomer in the C1 conformation, i.e. all the hydroxyl groups will be equatorial in the β anomer. However, in the C1 conformation of β -D-altropyranose 6-phosphate HO-2 and HO-3 will be axial. α -D-Altropyranose, on the other hand, exists in both the 1C and C1 conformations in aqueous solution (Angyal, 1969) and in the 1C conformation HO-1, HO-2 and HO-3 will be equatorial, thus resembling the C1 conformation of D-glucopyranose. It is possible, therefore, that the α -anomer of D-altropyranose 6-phosphate binds to the enzyme in the 1C conformation.

Replacement of a ring hydroxyl group by a fluorine substituent does not result in loss of binding, since the deoxyfluoro-D-glucose 6-phosphates are all substrates. The K_m values, listed in Tables 1 and 2, for the derivatives for both yeast and rat liver are all of the

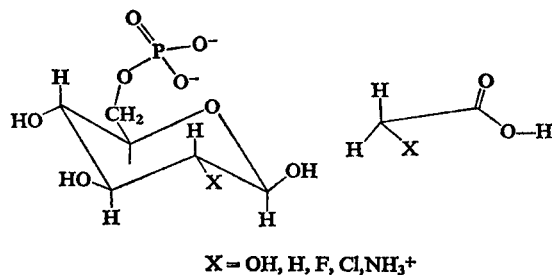


Fig. 6. C1 conformation of C-2 substituted β -D-glucopyranose 6-phosphates and the structure of α -substituted acetic acids

same order, but the relative V_{max} values are quite different. 3-Deoxy-3-fluoro-D-glucose 6-phosphate and 4-deoxy-4-fluoro-D-glucose 6-phosphate each have a high relative V_{max} value (0.12–0.40), but the C-2-substituted D-glucose 6-phosphates have widely different relative V_{max} values.

When the deoxyfluoro-D-glucopyranoses were examined as substrates for yeast hexokinase (Bessell *et al.*, 1972) it was found that, although these derivatives had different K_m values, all of them had high relative V_{max} values because the substitutions were made at a part of the molecule distant from the part involved in the reaction. However, with the D-glucose 6-phosphate derivatives, substitution at C-2 is substitution at the vicinal carbon to the carbon atom involved in the reaction. The large variation in the relative V_{max} values could be explained by the inductive effect of the C-2 substituent. If the hydrogen atom is removed from C-1 by hydride transfer in the dehydrogenation reaction an electron-withdrawing substituent at C-2 should retard the enzymic rate, whereas at C-3 and C-4 little effect would be expected.

The order of electronegativity of the C-2 substituents can be established from the dissociation constants of α -substituted aliphatic carboxylic acids (Kortüm *et al.*, 1961). The comparison between these compounds and C-2-substituted D-glucose 6-phosphates is shown in Fig. 6. The order is $NH_3^+ > F > Cl > OH > H$. If the effect of the C-2 substituent on the enzymic rate simply reflects electronegativity, then the order of V_{max} values should be $H > OH > Cl > F > NH_3^+$. For the yeast enzyme the order found experimentally is $OH > H > Cl > NH_3^+ > F$. The relative V_{max} value for 2-deoxy-D-arabino-hexose 6-phosphate is slightly lower than expected. This value, however, was only found by extrapolation, because inhibition was observed at high substrate concentrations. The relative V_{max} value for D-glucosamine 6-phosphate is higher than expected. Although D-glucosamine 6-phosphate exists in aqueous solution in a zwitterionic form, it may not do so completely in the active site of the

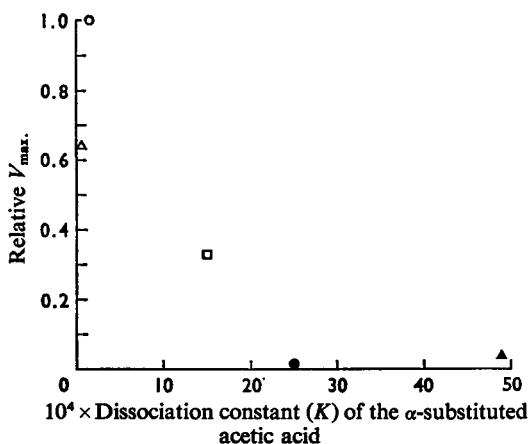


Fig. 7. Correlation between the relative V_{\max} for some C-2-substituted D-glucose 6-phosphates and the dissociation constants of the correspondingly substituted α -substituted acetic acids

For details see the text. The dissociation constants were obtained from Kortüm *et al.*, 1961. The substituents were: ○, OH; △, H; □, Cl; ●, F; ▲, NH₃⁺.

enzyme if the pK_a of the protonated amino group is lower (pK_a for the protonated amino group of 2-aminoethanol 1-phosphoric acids is 10.89 ± 0.014 ; Clarke *et al.*, 1955). A higher proportion of the D-glucosamine 6-phosphate molecules would then have a non-protonated amino group at pH 7.5 and a higher relative V_{\max} value than expected would be obtained. The correlation between the electronegativity of the C-2 substituent and the relative V_{\max} values for the yeast enzyme is shown in Fig. 7.

For the rat liver enzyme the order found experimentally is OH > Cl > H > F > NH₃⁺. Only the hydrogen substituent is out of order in this case, but as the relative V_{\max} value for 2-deoxy-D-arabino-hexose 6-phosphate is much lower than expected the effect of the C-2 substituents cannot solely be electronic and factors such as steric factors must be considered as well. Hydrogen and fluorine atoms are smaller than a hydroxyl group whereas a chlorine atom is approximately of the same size [covalent radii: H, 0.030 nm (0.30 Å); F, 0.064 nm (0.64 Å); Cl, 0.099 nm (0.99 Å); O+H, 0.096 nm (0.96 Å); Pauling, 1950]. This could explain the high relative V_{\max} value for 2-chloro-2-deoxy-D-glucose 6-phosphate.

The substrate inhibition observed with 2-deoxy-D-arabino-hexose 6-phosphate is noteworthy, since not only was this not observed with D-glucose 6-phosphate but the inhibition only occurred with the yeast enzyme.

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